Review Article

Mechanisms of Disease

FRANKLIN H. EPSTEIN, M.D., *Editor*

SINGLE-GENE MUTATIONS RESULTING IN REPRODUCTIVE DYSFUNCTION IN WOMEN

ELI Y. ADASHI, M.D., AND JON D. HENNEBOLD, PH.D.

FERTILITY in women is regulated by a series of highly coordinated and synchronized interactions in the hypothalamic–pituitary–ovarian axis (Fig. 1). The central regulator of the axis is the group of neurons that secrete gonadotropin-releasing hormone (GnRH).[1] Their cell bodies reside in the arcuate nucleus, and their exons terminate in the median eminence near the hypothalamic–pituitary portal vasculature. These neurons are unique in that they have an intrinsic firing frequency such that GnRH is secreted in pulses at 60-to-90-minute intervals into the portal vasculature to be conveyed to the anterior pituitary gland. There GnRH binds to specific cell-surface receptors on the gonadotrophs — that is, the pituitary cells that produce follicle-stimulating hormone and luteinizing hormone.[2] The secretion of follicle-stimulating hormone and luteinizing hormone is therefore also pulsatile.[2] The two gonadotropins stimulate terminal ovarian folliculogenesis, the process by which a cohort of secondary early antral follicles is recruited, only to be winnowed down to a single dominant follicle that releases its oocyte after the mid-cycle surge in luteinizing hormone secretion. The remnants of the follicle form the corpus luteum, which produces progesterone, a hormone central to implantation and early gestation.[3]

The operational characteristics of the reproductive axis leave little room for error, which may explain the relatively high incidence of cycling disorders in women. Most of these are thought to be acquired disorders of suprapituitary origin, but some women may be genetically predisposed to them. Most genetically determined cycling disorders are of subpituitary origin, with the ovary being the most common site of origin (Fig. 1).

Many of the genetically determined cycling disorders become apparent during puberty, when the physical and biochemical changes that lead to adult reproductive function occur. Pubertal development begins with the initiation of pulsatile secretion of GnRH by the hypothalamus, which in turn activates the pituitary–ovarian axis, leading to the production of estrogen by the ovaries, development of secondary sex characteristics, and initiation of menstrual cycles.

A molecular basis has been established for 11 genetically determined and phenotypically apparent cycling disorders (Table 1). Ten of these disorders are due to loss-of-function mutations, and one is due to a gain-of-function mutation affecting the α-subunit of the stimulatory G protein (Gsα) involved in the adenylyl cyclase–cyclic AMP (cAMP) pathway of signal transduction in women with the McCune–Albright syndrome. The inheritance of most of these disorders is autosomal recessive. Fewer than 200 cases of these disorders have been reported, but there have been no systematic prospective studies to determine their frequency among women with cycling disorders.

Not all genetically determined cycling disorders occur as the result of an intrinsic abnormality of the hypothalamic–pituitary–ovarian axis. Most notable in this regard is congenital adrenal hyperplasia caused by 21-hydroxylase deficiency (due to a *CYP21* mutation) or 11β-hydroxylase deficiency (due to a *CYP11B1* mutation), which in their classic forms are associated with ambiguous genitalia and various degrees of virilization (Fig. 2A).[4,5] Both have been the subject of other reviews and are beyond the scope of this article.[6,7] Other forms of congenital adrenal hyperplasia in which extraadrenal steroidogenesis is affected are considered here (Fig. 2B).

There are genetic abnormalities in the hypothalamic–pituitary–ovarian axis that do not cause phenotypic abnormalities in females. One is a dominantly inherited gain-of-function mutation in the luteinizing hormone receptor, which in males is associated with premature activation of the reproductive axis ("testotoxicosis").[8-12] Mutations of 17β-hydroxysteroid dehydrogenase type 3, which cause pseudohermaphroditism in males,[13] do not affect females, because this enzyme is expressed only in the testes.[14,15] Missense mutations in the luteinizing hormone β-sub-

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From the Division of Reproductive Sciences, Department of Obstetrics and Gynecology, University of Utah Health Sciences Center, Salt Lake City. Address reprint requests to Dr. Adashi at the Department of Obstetrics and Gynecology, University of Utah Health Sciences Center, 546 Chipeta Way, Rm. 109, Salt Lake City, UT 84108, or at eadashi@hsc.utah.edu.

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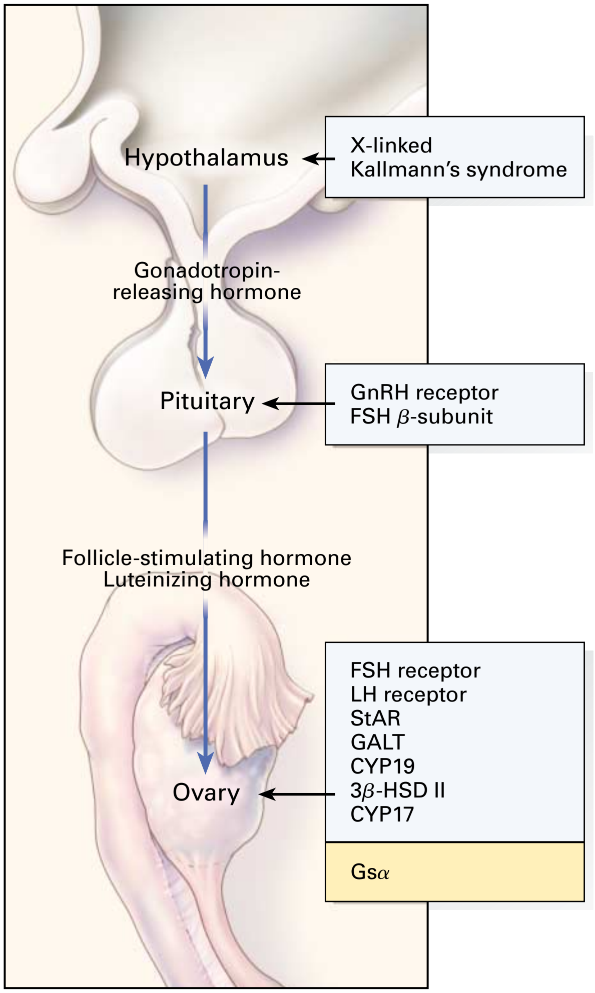

Figure 1. The Hypothalamic–Pituitary–Ovarian Axis and the Single-Gene Mutations That Are Responsible for Compromising Female Reproduction.

The blue background indicates loss-of-function mutations, and the yellow background a gain-of-function mutation. GnRH denotes gonadotropin-releasing hormone, FSH follicle-stimulating hormone, LH luteinizing hormone, StAR steroidogenic acute regulatory protein, GALT galactose-1-phosphate uridyltransferase, CYP19 aromatase, 3β-HSD II 3β-hydroxysteroid dehydrogenase type II, CYP17 17α-hydroxylase and 17,20-lyase, and Gsα the α-subunit of the stimulatory G protein.

Table 1. Incidence and Mode of Transmission of Single-Gene Mutations Associated with Reproductive Dysfunction in Women.

PHENOTYPE	GENE	INCIDENCE	MODE OF TRANSMISSION
Kallmann's syndrome	*KAL*	1 in 50,000	Autosomal recessive or autosomal dominant
Gonadotropin-releasing hormone resistance	*GNRHR*	Rare	Autosomal recessive
Isolated follicle-stimulating hormone deficiency	*FSHB*	Rare	Autosomal recessive
Hypergonadotropic hypogonadal ovarian failure	*FSHR*	1 in 8300 (Finland)	Autosomal recessive
Luteinizing hormone resistance	*LHR*	Rare	Autosomal recessive
Congenital lipoid adrenal hyperplasia	*STAR*	Rare	Autosomal recessive
Galactosemia	*GALT*	1 in 187,000	Autosomal recessive
McCune–Albright syndrome	*GNASI*	Rare	Dominant postzygotic mutation
Aromatase deficiency	*CYP19*	Rare	Autosomal recessive
3β-Hydroxysteroid dehydrogenase type II deficiency	*HSD3B2*	Rare	Autosomal recessive
17α-Hydroxylase deficiency	*CYP17*	Rare	Autosomal recessive

unit gene have been described that result in the secretion of a luteinizing hormone that has a shorter half-life in vivo and greater bioactivity in vitro than the wild-type hormone, but it is not associated with clinical abnormalities.[16]

KALLMANN'S SYNDROME

Kallmann's syndrome is characterized by an embryonic failure of migration of both the olfactory neurons and the GnRH-producing neurons.[17-22] Normally, the GnRH-producing neurons, which originate in the medial olfactory placode, cross the nasal septum and establish axodendritic synapses with developing cells of the forebrain.[23] Without these synaptic connections, the neurons do not aggregate in the hypothalamus, and the olfactory bulbs and tracts are not formed. The characteristic feature of Kallmann's syndrome is hypogonadotropic hypogonadism associated with anosmia.[24]

This syndrome occurs in both sexes but is five times as common in men as in women.[22] In most affected families, the syndrome is inherited as an X-linked recessive trait, so that affected males are encountered in every other generation. An affected female must be homozygous, an outcome possible only through the successful union of an affected man and a carrier woman. Since affected men are infertile and GnRH-replacement therapy has become available only during the past two decades, the likelihood that an affected female inherited the syndrome as an X-linked recessive trait is very low. It follows that in most affected females the disorder is inherited as an autosomal recessive or dominant trait.

In 1991 the cloning of a putative "Kallmann" gene on the distal end of the short arm of the X chromosome was reported. Subsequently, missense and nonsense mutations and partial and complete deletions of this gene have been identified in different families. The putative Kallmann gene encodes a 679-amino-acid protein whose proposed structure suggests it is an adhesion molecule involved in embry-

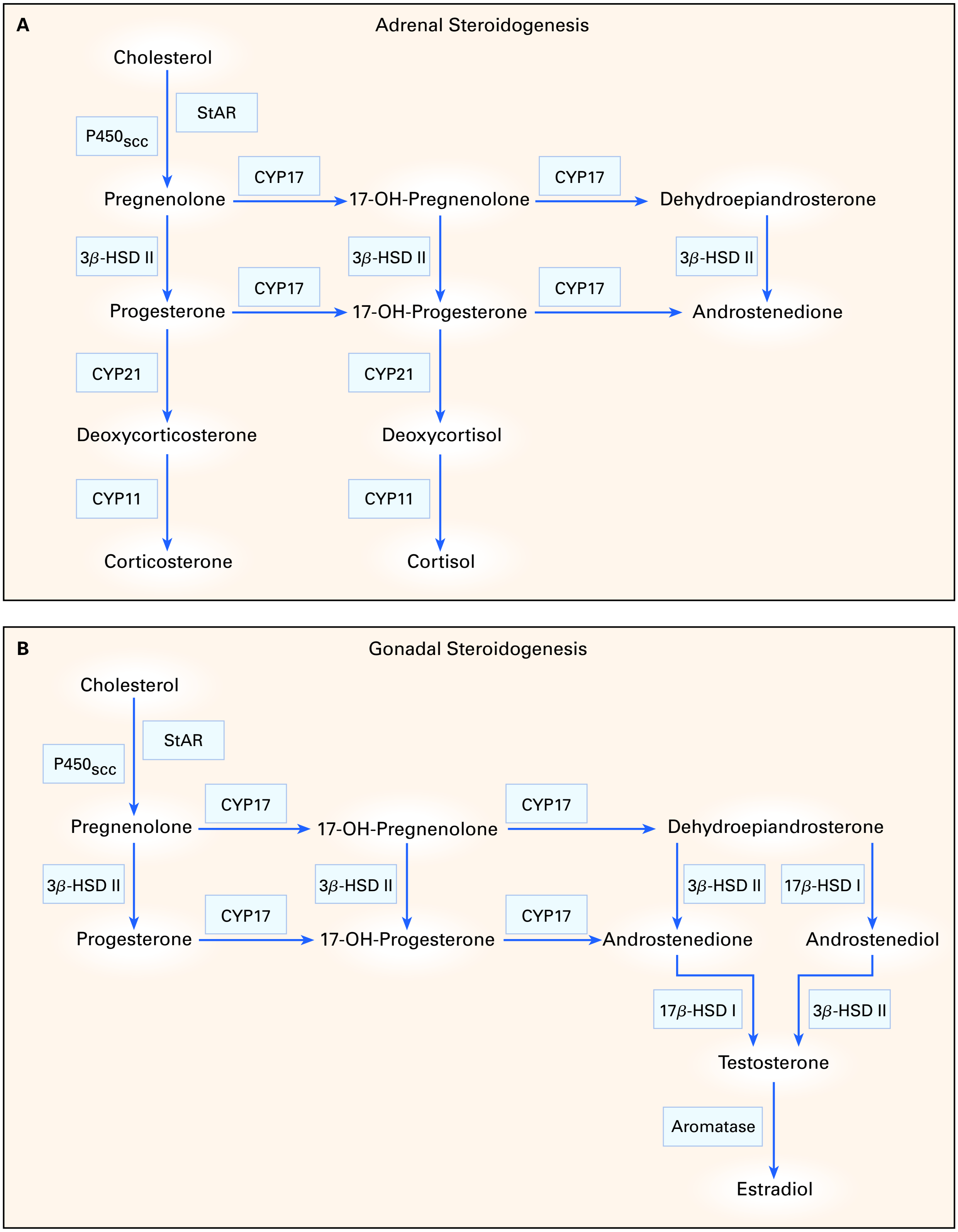
A
Adrenal Steroidogenesis
Cholesterol
StAR
P450scc
Pregnenolone
CYP17
17-OH-Pregnenolone
CYP17
Dehydroepiandrosterone
3β-HSD II
3β-HSD II
3β-HSD II
Progesterone
CYP17
17-OH-Progesterone
CYP17
Androstenedione
CYP21
CYP21
Deoxycorticosterone
Deoxycortisol
CYP11
CYP11
Corticosterone
Cortisol
B
Gonadal Steroidogenesis
Cholesterol
StAR
P450scc
Pregnenolone
CYP17
17-OH-Pregnenolone
CYP17
Dehydroepiandrosterone
3β-HSD II
3β-HSD II
3β-HSD II
17β-HSD I
Progesterone
CYP17
17-OH-Progesterone
CYP17
Androstenedione
Androstenediol
17β-HSD I
3β-HSD II
Testosterone
Aromatase
Estradiol

Figure 2. Adrenal (Panel A) and Gonadal (Panel B) Steroidogenic Pathways.

StAR denotes steroidogenic acute regulatory protein, CYP17 17α-hydroxylase and 17,20-lyase, 3β-HSD II 3β-hydroxysteroid dehydrogenase type II, CYP21 21-hydroxylase, CYP11 11β-hydroxylase, and 17β-HSD I 17β-hydroxysteroid dehydrogenase type I.

onic neuronal migration.[25,26] The absence of the protein in question precludes the olfactory neurons and the GnRH-producing neurons from reaching their destination. The autosomal genes have not been identified.

RESISTANCE TO GONADOTROPIN-RELEASING HORMONE

No abnormality of the GnRH gene has yet been identified in patients with idiopathic hypogonadotropic hypogonadism. However, a mutation of the GnRH-receptor gene was recently identified in a kindred with idiopathic hypogonadotropic hypogonadism.[27] The 37-year-old propositus had amenorrhea and infertility after a single episode of spontaneous uterine bleeding at the age of 18 years. Spontaneous thelarche occurred at the age of 14 years. Her serum gonadotropin and estradiol concentrations were in the low-normal range for the early follicular phase of the menstrual cycle.

The proband and her brother, who also had hypogonadism, had different mutations of each parental allele of the GnRH-receptor gene (Gln106Arg and Arg262Gln substitutions).[27] The parents were phenotypically normal, and each was heterozygous for one of the mutations. A normal sister was heterozygous for the paternal mutation.

Cells were transfected with the wild-type and mutant receptor complementary DNA (cDNA). In cells expressing the Gln106Arg substitution, localized to the first extracellular loop of the receptor, the level of GnRH binding and stimulation of cellular phospholipase C activity was markedly lower than in cells expressing wild-type receptors. In cells expressing the Arg262Gln substitution, localized to the third intracellular loop, hormone binding was normal, but phospholipase C activity did not increase.

The phenotype of the proband raises the possibility that other women with lesser degrees of reproductive dysfunction might have mutations of the GnRH receptor that result in a less marked impairment of receptor function.

ISOLATED DEFICIENCY OF FOLLICLE-STIMULATING HORMONE

The first woman who proved to have isolated deficiency of follicle-stimulating hormone presented in 1972 with primary amenorrhea, sexual infantilism, and a eunuchoid habitus.[28,29] Serum luteinizing hormone concentrations were high, and serum follicle-stimulating hormone and estradiol concentrations were very low. Administration of human menopausal gonadotropins for 14 days resulted in progressive increases in serum estradiol concentrations and cervical mucus secretion (an estrogenic end point). After administration of human chorionic gonadotropin on day 14 of the simulated cycle, there was a shift in basal body temperature and a rise in serum progesterone concentrations indicative of ovulation, which was in due course followed by conception.[30]

Twenty-one years later, the molecular basis of isolated deficiency of follicle-stimulating hormone was found to be a homozygous deletion in codon 61 of the gene for the β-subunit of follicle-stimulating hormone, a finding predicted to result in the truncation of the protein.[31] On the basis of established structure–function relations, the truncated protein product was predicted to lack key segments essential for associating with the α-subunit, so that little, if any, follicle-stimulating hormone would be formed, thereby explaining the absence of immunologic and biologic activity. A 14-day course of treatment with purified human follicle-stimulating hormone alone resulted in ovulation and conception.[31] Another woman with similar clinical findings had different mutations in the two alleles of the gene for the β-subunit of follicle-stimulating hormone.[32] One allele had the same premature stop mutation at codon 61, and the other allele had a conversion of thymidine to guanine in codon 51 of exon 3, which leads to a substitution of glycine for cysteine.

These results demonstrate that congenital deficiency of follicle-stimulating hormone does not result in permanent impairment of ovarian development and that follicle-stimulating hormone is indispensable only during the last 14 days of the follicular phase of the cycle.

HYPERGONADOTROPIC HYPOGONADISM

In 1995 a cycling disorder attributable to mutations of the gene for the follicle-stimulating hormone receptor was described.[33] This discovery resulted from a concerted effort to identify the genetic determinants of hypergonadotropic hypogonadism in women by using a registry from a network of university hospitals in Finland. The inclusion criteria were primary amenorrhea or secondary amenorrhea with onset before the age of 20 years, a 46,XX karyotype, serum follicle-stimulating hormone concentrations greater than 40 mIU per milliliter (indicative of ovarian failure), a date of birth between 1950 and 1976, and no other known cause of ovarian failure, such as surgery, chemotherapy, or radiation therapy. Among 3856 women in the registry, 75 met the inclusion criteria. Fifty-seven were the only affected women in their families, and 18 had similarly affected sisters.

A conventional linkage analysis performed on 36 women from six multiplex families localized the trait to the short arm of chromosome 2, a region containing the genes for both the luteinizing hormone receptor and the follicle-stimulating hormone receptor.[33] Analysis of the follicle-stimulating hormone–receptor gene revealed that 29 of the affected women studied had a single missense mutation (a conversion of cytosine to thymidine) at position 566 in exon 7 of this gene. This mutation is in the extracellular domain of the receptor and could lead to disruption of hormone binding (Fig. 3A). In cells transfected with mutant follicle-stimulating hormone–receptor cDNA, binding of follicle-stimulating hormone and stimulation of cAMP production were lower in cells transfected with wild-type receptor cDNA. In several affected women, ovarian biopsy revealed a normal complement of primordial follicles, thereby ruling out premature ovarian failure.[34] These findings confirm that exposure to follicle-stimulating hormone is not necessary for normal ovarian development.

A somatic mutation of the follicle-stimulating hormone receptor was recently found in 9 of 13 ovarian sex-cord tumors and 2 of 3 small-cell carcinomas of the ovary.[35] The mutation was a heterozygous conversion of thymine to cytosine at nucleotide 1777 (codon 591) that changed a phenylalanine to a serine residue and resulted in loss of cellular sensitivity to follicle-stimulating hormone. How such a change in cell function contributes to tumor formation or growth is not known.

RESISTANCE TO LUTEINIZING HORMONE

A cycling disorder attributable to mutations of the luteinizing hormone–receptor gene was first reported in 1996. The propositus presented with prolonged periods of amenorrhea, punctuated by irregular episodes of apparently anovulatory uterine bleeding.[36] Laboratory studies revealed slightly elevated serum gonadotropin concentrations and normal serum estradiol and progesterone concentrations. Her two brothers had male pseudohermaphroditism and Leydig-cell hypoplasia.[37] All three had a single-nucleotide missense mutation (conversion of guanine to cytosine) at position 1787 of the luteinizing hormone–receptor gene, which led to an Ala593Pro substitution in the third extracellular loop of the receptor (Fig. 3B).[36,37] The father was heterozygous for this mutation, indicating autosomal recessive transmission. Studies of cells transfected with cDNA of the luteinizing hormone receptor revealed that the binding and biologic activity of chorionic gonadotropin were markedly reduced in cells transfected with mutant cDNA as compared with cells transfected with wild-type cDNA. The mutation may affect the intracellular processing of the luteinizing hormone receptor, thereby resulting in limited translocation of the mature protein to the plasma membrane.

An unrelated woman with prolonged amenorrhea after a single episode of vaginal bleeding[38] had a mutation in the luteinizing hormone–receptor gene that led to the introduction of a stop codon at position 1660 (Fig. 3B). The resultant protein was truncated in the third cytoplasmic loop. Cells transfected with mutant receptor cDNA did not bind luteinizing hormone or respond to the hormone with an in-

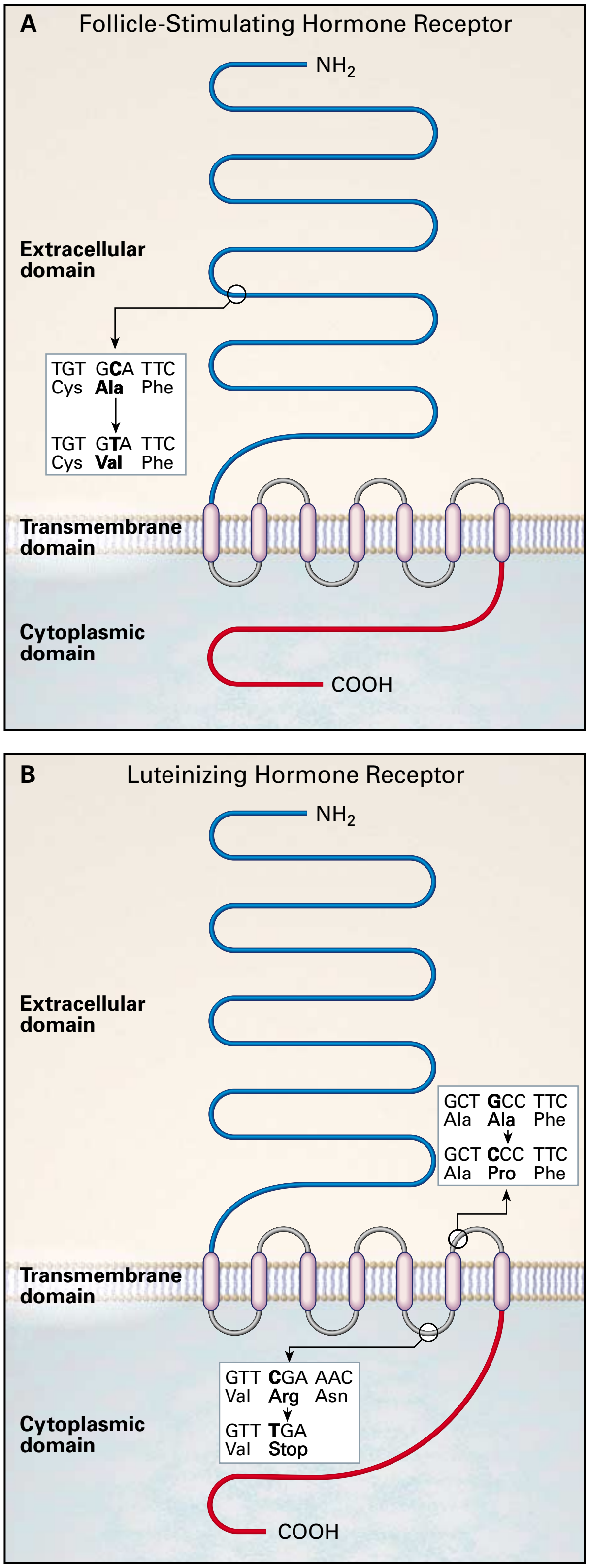
A
Follicle-Stimulating Hormone Receptor
NH_2
Extracellular domain
TGT GCA TTC
Cys Ala Phe
TGT GTA TTC
Cys Val Phe
Transmembrane domain
Cytoplasmic domain
COOH
B
Luteinizing Hormone Receptor
NH_2
Extracellular domain
GCT GCC TTC
Ala Ala Phe
GCT CCC TTC
Ala Pro Phe
Transmembrane domain
GTT CGA AAC
Val Arg Asn
GTT TGA
Val Stop
Cytoplasmic domain
COOH

Figure 3. Structure of the Follicle-Stimulating Hormone Receptor (Panel A) and the Luteinizing Hormone Receptor (Panel B) and the Associated Location and Type of Characterized Mutations.

crease in cAMP production. Thus, deletion of the region of the receptor that encompasses not only the sixth and seventh transmembrane segments but also a cytoplasmic loop results in loss of receptor activity. This clinical entity must be considered in the differential diagnosis of primary and secondary amenorrhea.

CONGENITAL LIPOID ADRENAL HYPERPLASIA

The steroidogenic acute regulatory protein is a mitochondrial phosphoprotein responsible for translocating cholesterol from the outer to the inner mitochondrial membrane,[39] where it is converted to pregnenolone by the cholesterol side-chain cleavage enzyme ($P450_{scc}$) (Fig. 2). This is the first committed step in the steroidogenic cascade. Thus, steroidogenic acute regulatory protein is a rate-limiting protein in steroidogenesis, and a loss of its activity would be expected to result in defective adrenal and gonadal steroidogenesis. That this is the case has been documented in congenital adrenal lipoid hyperplasia, a condition attributable to loss-of-function mutations in the gene for steroidogenic acute regulatory protein.[40,41]

Female patients with congenital adrenal lipoid hyperplasia who receive glucocorticoid and mineralocorticoid supplementation (who otherwise die in infancy as a result of adrenal insufficiency) have normal pubertal development, including thelarche, pubarche, and menarche, but then have ovarian failure.[42-44] The ovarian failure may occur after puberty because it is only then that gonadal steroidogenesis is sufficiently active to cause accumulation of enough cholesterol to damage ovarian cells.[45] Among three patients, analysis of DNA revealed a homozygous nonsense stop mutation in codon 258 (exon 7) in one patient and the deletion of a single nucleotide in codon 238 in the other allele in the other two patients. The deletion resulted in loss of a stop codon and extension of the protein by 34 nonsense amino acids. Transfection studies of the latter mutation revealed limited, if any, activity of steroidogenic acute regulatory protein.

GALACTOSEMIA

Galactosemia results in hepatomegaly, jaundice, and failure to thrive soon after birth. It is caused by a deficiency of galactose-1-phosphate uridyltransferase. This enzyme catalyzes the conversion of galactose-1-phosphate to uridyl-dephosphogalactose, which enters the pathway of glucose metabolism.[46] Additional clinical manifestations include cataracts and mental retardation, as well as reproductive failure in a substantial percentage (62 percent) of affected women, but not men.[47,48] More than 12 mutations have been described in the *GALT* gene, which codes for galactose-1-phosphate uridyltransferase.[49]

Women with galactosemia may have primary or secondary amenorrhea despite a lifelong galactose-free diet.[50,51] Ovarian biopsies in women with galactosemia reveal few primordial follicles, numerous atretic follicles, and the complete absence of intermediate or graafian follicles.[51] In contrast, an autopsy of a five-day-old girl with galactosemia revealed abundant oocytes.[50] Whether the ovarian failure is caused by the accumulation of galactose-1-phosphate or a deficiency of downstream metabolites is not known.

McCUNE–ALBRIGHT SYNDROME

The McCune–Albright syndrome is characterized by patchy cutaneous hyperpigmentation (café au lait spots), polyostotic fibrous dysplasia, and several endocrine disorders, including toxic multinodular goiter, pituitary gigantism, amenorrhea–galactorrhea, Cushing's syndrome, and precocious puberty. The last can occur at any time from a few months after birth to late childhood. The precocious puberty is gonadotropin-independent and cannot be arrested by treatment with a GnRH analogue.[52,53]

Analysis of the distribution of the cutaneous hyperpigmentation led to the hypothesis that the McCune–Albright syndrome is attributable to a somatic mutation that occurs early in embryogenesis, thereby resulting in a mosaic pattern of expression.[54] The absence of familial cases (except in monozygotic twins) provided further support for this hypothesis.[55]

The defect underlying the syndrome was identified as mutations (Arg201His or Arg201Cys) within exon 8 of the *Gsα* gene.[56-58] These mutations result in constitutive activation of the encoded Gsα protein. Given that the action of gonadotropins is mediated by Gsα, the precocious puberty in patients with the McCune–Albright syndrome is probably due to constitutive activation of gonadotropin signaling (Fig. 4). These same mutations are found in some autonomously functioning thyroid adenomas and growth hormone–secreting pituitary adenomas.[59]

The hypothesis that the mutations are somatic and expressed in a mosaic fashion predicts interorgan and intraorgan heterogeneity. To address this point, ovarian-biopsy specimens composed of both histologically normal and histologically abnormal (luteinized) cellular elements were analyzed for the presence of *Gsα* mutations.[56] The normal ovarian tissue contained only the wild-type sequence, whereas the abnormal luteinized tissue contained both mutant and wild-type sequences.

AROMATASE DEFICIENCY

The possibility of reproductive dysfunction attributable to aromatase deficiency was first proposed in 1991 on the basis of clinical observations in a woman with progressive virilization during the third trimester of pregnancy; very low serum estradiol, estrone, and estriol concentrations; and high serum testosterone concentrations.[60] To assess the possibility of placental aromatase deficiency, the mother re-

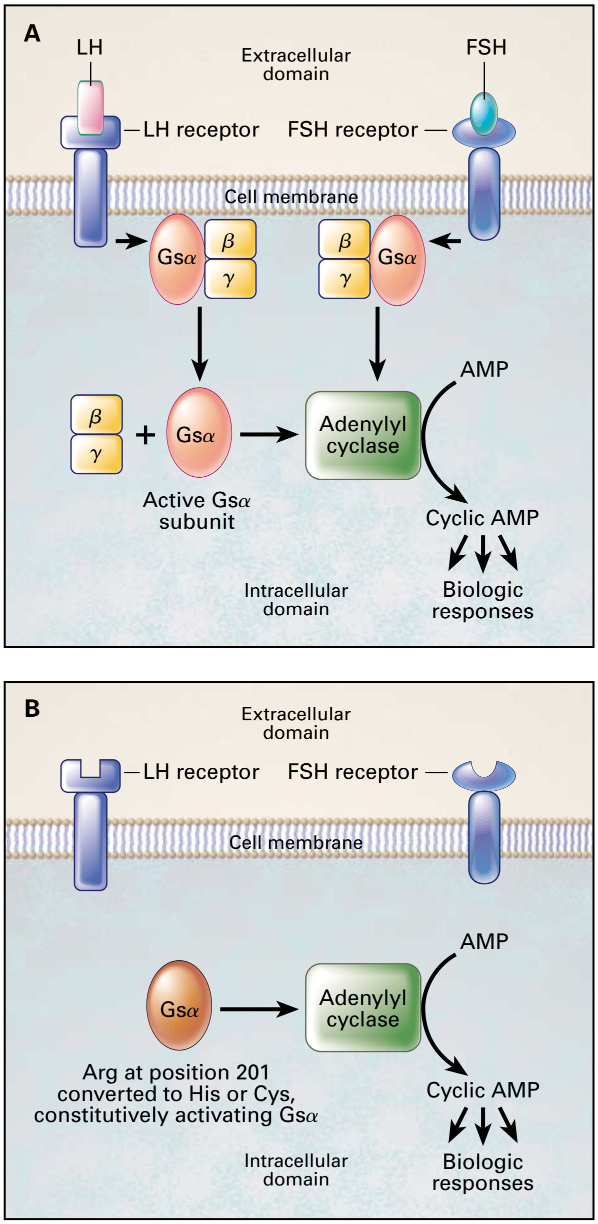
A
LH
Extracellular domain
FSH
LH receptor
FSH receptor
Cell membrane
Gsα
β
γ
β
γ
Gsα
AMP
β
γ
+
Gsα
Adenylyl cyclase
Active Gsα subunit
Cyclic AMP
Intracellular domain
Biologic responses
B
Extracellular domain
LH receptor
FSH receptor
Cell membrane
AMP
Gsα
Adenylyl cyclase
Arg at position 201 converted to His or Cys, constitutively activating Gsα
Cyclic AMP
Intracellular domain
Biologic responses

Figure 4. Gonadotropin-Receptor Signaling in Normal Subjects (Panel A) and Patients with the McCune–Albright Syndrome (Panel B).

Binding of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) to their receptors normally activates adenylyl cyclase through the action of the α-subunit of the stimulatory G protein (Gsα). Activating mutations in the *Gsα* gene result in a constitutively active protein that stimulates adenylyl cyclase activity in the absence of interactions between gonadotropins and their receptors.

ceived an infusion of dehydroepiandrosterone sulfate, an androgenic substrate of placental aromatase. There was no increase in serum estradiol, estrone, and estriol concentrations or the urinary excretion of estrogenic metabolites, whereas these values increased in normal pregnant women. The newborn infant, a girl with a 46,XX karyotype, had clitoromegaly and a urogenital sinus, suggesting exposure to androgens in the first trimester of pregnancy. The molecular basis of the disorder proved to be a missense mutation involving a single nucleotide in intron 6 that removed a splicing site, resulting in the addition of 87 nucleotides.[61,62] The resultant protein contained a nonsensical insert of 29 amino acids. In cells transfected with the mutant aromatase cDNA, there was markedly less aromatase activity than in cells transfected with its wild-type counterpart.

An 18-year-old woman with aromatase deficiency has been described.[63] She had ambiguous external genitalia at birth; no adrenal cause was identified. Laparotomy at 17 months of age revealed normal internal female genitalia. A cortical ovarian biopsy disclosed an apparently normal complement of primordial follicles. At the age of 18, she had primary amenorrhea, sexual infantilism, and clitoromegaly. Laboratory studies revealed a normal female karyotype, high serum testosterone and androstenedione concentrations, undetectable serum estradiol and estrone concentrations, and moderately high serum follicle-stimulating hormone and luteinizing hormone concentrations. Pelvic imaging disclosed multiple ovarian cysts.

In two other reports of women with aromatase deficiency, the possibility of aromatase deficiency was raised by the observation of striking gestational virilization that regressed post partum.[64,65] The women had low serum estrogen concentrations and high serum androgen concentrations. At birth, one had masculinized external genitalia, including clitoromegaly, and the other had complete posterior fusion compatible with the formation of a urogenital sinus. At six months of age, ultrasonography in one child revealed enlarged ovaries.[65] At two years of age, the ovaries were still enlarged, and laparoscopy revealed multiple ovarian cysts composed of numerous large antral follicles. Hormonal studies revealed hyperandrogenism, hypoestrogenism, and hypergonadotropism. Administration of estradiol decreased serum gonadotropin concentrations and the size of the ovaries, suggesting that the cystic ovarian enlargement was gonadotropin-dependent. The other child, at 12 years of age, also had ovarian enlargement and similar biochemical findings.[64]

Thus, aromatase deficiency results in nonadrenal female pseudohermaphroditism characterized by virilized external genitalia at birth and by primary amenorrhea, sexual infantilism, eunuchoid proportions, tall stature, hyperandrogenism, hypoestrogenism, hypergonadotropism, and multicystic ovaries in adolescence.[66] The hyperandrogenism probably results from the disruption in the conversion of androgens to estrogens, and the resulting hypergonadotropism causes the multicystic ovaries. The findings document the important role of placental aromatase in disposing of maternal and fetal androgens and thus in protecting the mother and the infant from virilization. They also indicate that the congenital absence of aromatase is compatible with normal ovarian development and that aromatase deficiency must be considered in the differential diagnosis of primary amenorrhea as well as in women with gestational and nongestational hyperandrogenism.

CONGENITAL ADRENAL HYPERPLASIA CAUSED BY DEFICIENCY OF 3β-HYDROXYSTEROID DEHYDROGENASE TYPE II

Congenital adrenal hyperplasia, the most frequent cause of adrenal insufficiency in newborns, denotes a group of disorders resulting from inherited dysfunction in any one of the steps of cortisol synthesis (Fig. 2).[67] Pathologically, congenital adrenal hyperplasia is characterized by hyperplastic growth of the adrenal glands due to increased corticotropin secretion. Deficiency of 3β-hydroxysteroid dehydrogenase is the second most common cause of congenital adrenal hyperplasia, accounting for about 10 percent of cases.[67,68] In contrast to 21-hydroxylase deficiency and 11β-hydroxylase deficiency, which affect only adrenal function, 3β-hydroxysteroid dehydrogenase deficiency affects both adrenal and gonadal function (Fig. 2). Transmitted as an autosomal recessive trait, 3β-hydroxysteroid dehydrogenase deficiency is characterized by variable impairment of enzymatic activity and, consequently, variable clinical severity. Newborn infants with severe 3β-hydroxysteroid dehydrogenase deficiency have symptoms of both cortisol and aldosterone deficiency, which may be fatal if not diagnosed and treated early. Affected girls have either normal sexual development or mild virilization, which is most likely to be detected at puberty. Some patients present with chronic anovulation and even primary amenorrhea.[69] Although the precise pathophysiology of the cycling disorder is unknown, it is likely to be due to hyperandrogenism.

Systemic 3β-hydroxysteroid dehydrogenase deficiency should be incompatible with the synthesis and, therefore, the accumulation of androgens. However, there are two 3β-hydroxysteroid dehydrogenase isoenzymes.[70,71] The type I enzyme is predominantly expressed in the placenta and in peripheral tissues such as the skin and mammary glands, whereas the type II enzyme is predominantly expressed in the adrenal glands and the gonads. Point mutations have thus far been detected only in the gene for the type II enzyme.[72] Thus, the finding of normal type I enzyme activity in patients with type II deficiency appears to account for the hyperandrogenic state, by serving to catalyze the conversion of Δ^5 steroids such as dehydroepiandrosterone to Δ^4 steroids such as androstenedione and testosterone. The pubertal worsening of the hyperandrogenic state may be attributable to the activation of ovarian steroidogenesis and thus to the generation of increased quantities of Δ^5 steroids.

Nonclassic (adult onset) 3β-hydroxysteroid dehydrogenase deficiency has been diagnosed with increasing frequency in women with hyperandrogenism. However, sequencing of both the type I and the type II 3β-hydroxysteroid dehydrogenase genes in six patients revealed no mutations.[73] A mutation may exist in a gene encoding a protein that regulates the expression or the activity of 3β-hydroxysteroid dehydrogenase type II.

CONGENITAL ADRENAL HYPERPLASIA CAUSED BY 17α-HYDROXYLASE AND 17,20-LYASE DEFICIENCY

Congenital adrenal hyperplasia caused by 17α-hydroxylase deficiency (due to *CYP17* mutations) is a disorder characterized by marked impairment in the synthesis of glucocorticoid, androgen, and estrogen (Fig. 2). Fewer than 200 patients but over 20 different missense or nonsense mutations, as well as small insertions or deletions that alter the reading frame of the gene, have been reported.

Abnormalities in the *CYP17* gene affect both adrenal and gonadal steroidogenesis. Uniquely, however, the compensatory increase in corticotropin secretion leads to overproduction of mineralocorticoid intermediates, which causes hypertension and hypokalemia. The activity of both 17α-hydroxylase and 17,20-lyase is affected, because the *CYP17* gene encodes a single protein that can catalyze both reactions.[74]

Although women with 17α-hydroxylase deficiency have sexual infantilism and hypergonadotropic hypogonadism, their ovaries contain normal numbers of follicles, which reach the antral stage.[75] However, preovulatory follicles have not been identified, and substantial follicular atresia has been noted. Many of the women have multicystic ovaries. The hypergonadotropism, caused by estrogen deficiency, may be causally related to the induction of multicystic ovaries, such as occurs in women with aromatase deficiency.

CONCLUSIONS

The mutations identified to date that result in female reproductive dysfunction have been predominantly subpituitary in origin and are manifested primarily at puberty. The majority are rare autosomal recessive mutations that result in a loss of function. The frequency of detection of these mutations may increase as more prospective studies are performed and as a genetic basis is sought in more women with reproductive dysfunction.

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